Basic Science/Medicine

Plenary Lectures

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TUMOR SUPPRESSOR GENES

George Klein, Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden

(The statements in this abstract are based on work from a large number of laboratories. References will be supplied on request).

The rapidly growing field of tumor suppressor genes followed the development of the oncogene field with a delay of more than a decade. The concept that tumor development is an irreversible, one way process has been a dominating idea for a long time. Evidence of tumor - reverting phenotypic switches and, more recently, of specific genes that can counteract tumor development - designated by various names like anti-oncogenes, tumor suppressor genes, or emerogenes - emerged from several conceptually and experimentally independent sources. They can be briefly summarized as follows :

1. Downregulation of **temperature sensitive** v-src and v-erb B oncogenes at a non-permissive temperature for a short time has led to the irreversible differentiation of originally undifferentiated tumor cells. This was shown for cells of the myoblastic, chondroblastic, melanoblastic and erythroblastic lineages. This indicates that a precarious balance exists between the differentiation-blocking effect of these oncogens, and the structural or regulatory genes of differentiation. The differentiation block is thus not necessarily irreversible and can be overcome by shifting the balance between the expression of tumorgenic and tumor-antagonizing genes.
2. **Fusion** of normal with malignant cells has suppressed tumorgenicity in the derived somatic cell hybrids, as long as a fairly complete chromosome set was maintained from the normal parent. High-tumorgenic segregants arose by chromosome loss. Identification of normal-parent derived chromosome pairs that were regularly lost from the high malignant segregants has permitted the tentative localization of suppressor carrying chromosomes. In vivi inoculation of the chromosomally complete immortalized, but non-tumorgenic hybrids has led to the differentiation of the cells according to the program of the normal parent in certain combinations.
3. Using "suicidal selection" methods with BUDR and UV light, or high doses of radioactive thymidine, transformed cells could be selectedly killed in confluent cultures, while **revertants** that had regained contact inhibition, survived selectively. Revertants could be also selected on the basis of their higher resistance to certain toxic genes, e.g. ouabain. Revertants of the "post oncogene" type are particularly interesting. They maintain full expression of the unchanged transforming gene - usually activated ras, occasionally other oncogenes - but are morphologically non-transformed and often non-tumorgenic. Studies on the susceptibility of revertants, isolated from Ki-ras transformants, to retransformation by a spectrum of activated oncogenes has indicated that reversion may occur by a variety of routes, probably relatable to the action of different genes. Some reversion - inducing genes have now been defined, with K-rev-1, as ras-homologue and antagonist as the foremost example. Similarly antagonistic effects have been detected in other combinations. The proto-oncogene c-erbB inhibits the transforming effect of v-erbB. A non-transforming mutant of c-src, can inhibit the phenotypic effect of a transforming mutant.
4. **The Retinoblastoma** (Rb) gene contributes to the development of retinoblastomas and osteosarcomas **by ite loss**. Families with a germline mutation of Rb develop both tumors at a high rate, due to the loss of the normal allele during somatic development. Non-disjunction is the most frequent mechanism for this second loss. Reconstitution of Rb-negative cells with the Rb gene also appears to contribute to the development and/or progression of several solid tumors.
5. the p53 gene can function both as an oncogene and a tumor suppressor gene. Its loss appears to contribute to the development of several human and animal leukemias. The mutant form of the gene is transforming. mutations of p53 represent the most frequent currently known genetic change in some multi-cancer families with the Li-Fraumeni syndrome. Loss or mutation of p53 represents the second genetic change in the ostosarcomas that arise in Rb mutation carrying families (see above). Loss of one and mutation of the other allele is very common in colorectal sarcomas.
6. The transforming proteins of unrelated DNA tumor viruses like SV40, oncogenic adenoviruses and human papiloma viruses were found to target in on Rb and p53. This is a remarkable case of convergent evolution. SV40 large T uses two differnet domains to complex with Rb and p53 whereas adeno - and pamillomaviruses use two different proteins.

The normal function of Rb and p53 has not been clarified. Non-phofphorylated Rb protein may inhibit the entry of the cell into the S phase. Phosphorylation or complexing with the viral proteins mentioned inactivates this effect.

7. The membrane proteine LMP1, encoded by the **Epstein-Barr virus (EBV)**, can serve as a transforming gene or as a suppresor gene, depending on the cell in which it is expressed. It has a transforming effect for established lines of fibroblasts or epithelid cells. It suppresses the clonogenicity and tumorigenicity of EBV-negative Burkitt lymphoma (BL) cells.
8. Tumor cells that had developed through a series of genetic changes, e.g. colorectal carcinomas, can be reverted by wild type p53, known to compete with the resident, mutated p53 by heterodimerisation. Osteosarcoma cells that have lost both Rb and p53 but are primarily driven by the Ig/myc translocation, can be suppressed by wild type p53.

These examples suggest that it may not be necessary to "repair" a variety of genetic defects to suppress the tumorigenicity and/or growth of an autonomous tumor that has developed by multiple changes in several genes. Similar findings were made by introducing individual chromosomes, carrying appropriate suppressor genes, into tumor cells that have arisen by multiple genetic changes.

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GENES CODING FOR TUMOR REJECTION ANTIGENS. PERSPECTIVES FOR CANCER IMMUNOTHERAPY.

Boon T., van der Bruggen P., Lurquin C., Gaugler B., Brasseur F., De Plaen E., Van den Eynde B., Lethé B., Brichard V., Van Pel A., Coulie P.G. Ludwig Institute for Cancer Research, Brussels Branch, 74 Avenue Hippocrate - UCL 74.59, B-1200 Brussels, Belgium and Cellular Genetics Unit, Université Catholique de Louvain , Brussels, Belgium.

We have identified genes that code for antigens recognized on human tumors by cytolytic T lymphocytes (CTL) (1).

Human melanoma MZ2-MEL presents several distinct antigens recognized by autologous cytolytic T cells. The gene coding for one of these antigens, MZ2-E, has been isolated (2,3). This gene, named MAGE-1, belongs to a family of 12 closely related genes, that are located on chromosome X. The sequence of the gene in the melanoma tumor is identical to that found in normal tissues, but it is not expressed in normal tissues with the exception of testis. Gene MAGE-1 is expressed in approximately 40 % of melanoma tumors, on approximately 20% of breast tumors and 30% of non small cell bronchial tumors (4). Antigen MZ2-E consists of a peptide encoded by MAGE-1 that is presented by an HLA-A1 molecule. The sequence of the nonapeptide has been identified (5). Recently, we have found that gene MAGE-3 also codes for an antigen recognized by CTL on a HLA-A1 molecule. Gene MAGE-3 is expressed in 85% of melanoma tumors.

We have also identified two additional genes that code for antigens recognized by CTL on most melanomas of HLA-A2 patients (6, 7). The first gene codes for tyrosinase, the enzyme that synthesizes DOPA in the melanin pathway (8). This gene is expressed in melanoma and melanocytes. The second gene is unrelated to presently known sequences. Its expression is also restricted to melanoma and melanocytes.

The observations made with gene MAGE-1 may lead to new approaches of specific cancer immunotherapy. Patients can be typed readily for HLA-A1. The expression of gene MAGE-1 in their tumor can be assayed rapidly by reverse transcription and polymerase chain reaction (PCR) on the RNA of a small tumor sample. Positive patients can then be immunized with cells expressing antigen MZ2-E. Genetic constructs expressing high amounts of MAGE-1, HLA-A1 and interleukin 2 or 4 may provide improved immunogenicity. To establish whether immune responses will be generated, it will be necessary to compare CTL precursor frequencies in patients before and after immunization (9).

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